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Complete genome sequence of the halophilic bacterium *Spirochaeta africana* type strain (Z-7692^T) from the alkaline Lake Magadi in the East African Rift

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Spirochaeta africana Zhilina et al. 1996 is an anaerobic, aerotolerant, spiral-shaped bacterium that is motile via periplasmic flagella. The type strain of the species, Z-7692^T, was isolated in 1993 or earlier from a bacterial bloom in the brine under the trona layer in a shallow lagoon of the alkaline equatorial Lake Magadi in Kenya. Here we describe the features of this organism, together with the complete genome sequence, and annotation. Considering the pending reclassification of *S. caldaria* to the genus *Treponema*, *S. africana* is only the second 'true' member of the genus *Spirochaeta* with a genome-sequenced type strain to be published. The 3,285,855 bp long genome of strain Z-7692^T with its 2,817 protein-coding and 57 RNA genes is a part of the **Genomic Encyclopedia of Bacteria and Archaea** project.

Introduction

Strain Z-7692^T (= DSM 8902 = ATCC 700263) is the type strain of the species *Spirochaeta africana* [1]. The genus *Spirochaeta* currently consists of 18 validly named species [2]. The genus name was derived from the latinized Greek words 'speira' meaning 'a coil' and 'chaitê' meaning 'hair', yielding the Neo-Latin word 'Spirochaeta', a 'coiled hair' [2]. The species epithet was derived from the Latin word 'africana', of African continent, found in the African alkaline Lake Magadi [1]. Here we present a summary classification and a set of features for *S. africana* strain Z-7692^T, together with

the description of the complete genome sequencing and annotation.

Classification and features

16S rRNA analysis

A representative genomic 16S rRNA sequence of strain Z-7692^T was compared using NCBI BLAST [3,4] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [5] and the relative frequencies of taxa and keywords (reduced to their stem

[6]) were determined, weighted by BLAST scores. The most frequently occurring genera were *Spirochaeta* (91.1%), *Treponema* (5.8%) and *Cytophaga* (3.1%) (29 hits in total). Regarding the two hits to sequences from members of the species, the average identity within HSPs was 99.6%, whereas the average coverage by HSPs was 99.0%. Regarding the 19 hits to sequences from other members of the genus, the average identity within HSPs was 89.1%, whereas the average coverage by HSPs was 78.9%. Among all other species, the one yielding the highest score was *Spirochaeta asiatica* (NR_026300), which corresponded to an identity of 96.6% and an HSP coverage of 98.8%. (Note that the Greengenes database uses the INSDC (= EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification.) The highest-scoring environmental sequence was AF454308 (Greengenes short name 'spirochete clone ML320J-13'), which showed an identity of 90.6% and an HSP coverage of 99.3%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'microbi' (10.5%), 'mat' (8.8%), 'hypersalin' (6.3%), 'new' (4.2%) and 'world' (4.1%) (221 hits in total). Environmental samples which yielded hits of a higher score than the highest scoring species were not found, indicating that this species is rarely found in environmental sequencing.

Figure 1 shows the phylogenetic neighborhood of *S. africana* in a 16S rRNA based tree. The sequences of the three identical 16S rRNA gene copies in the genome differ by two nucleotides from the previously published 16S rRNA sequence (X93928).

Morphology and physiology

Cells of strain Z-7692^T are 0.25 to 0.3 µm in diameter and 15 to 30 µm (occasionally 7 to 40 µm) in length and form regular, stable primary coils [1] (Figure 2); spherical bodies were seen in stationary-phase cultures (not visible in Figure 2). The cells are motile by periplasmic flagella [1] (not visible in Figure 2). The cell mass is orange [1]. *S. africana* is a Gram-negative, anaerobic, aerotolerant, mesophilic microorganism (Table 1) with an optimal growth temperature between 30°C and 37°C, and no growth observed above 47°C [1]. The optimum pH is 8.8-9.8, no growth is observed at pH 8 or pH 10.8 [1]. *S. africana* is halophilic and does not grow at NaCl concentrations below 3% or above 10% (wt/vol) [1].

S. africana utilizes mainly mono- and disaccharides as carbon and energy sources. Amino acids cannot be fermented. Glucose is fermented to acetate, ethanol and H₂ as the main fermentation products, with a minor amount of lactate produced in stationary phase [1]. Strain Z-7692^T is able to ferment fructose, maltose, trehalose, saccharose, cellobiose, glucose, glycogen, starch. Poor growth was observed with mannose and or xylose, no growth with galactose, N-acetylglucosamin or ribose. A supplement of vitamins is required [1].

Chemotaxonomy

Major components detected in the fatty acid analysis are the fatty acids C_{14:0} (6.6%), C_{16:1cis9} (6.3%), C_{16:0} (19.0%), C_{18:1cis-9} (1.4%), summed feature 10 (C_{18:1cis11/trans9/trans6} and/or an unknown fatty acid with an equivalent chain length of 17.834) (34.9%), C_{18:0} (1.8%), C_{20:1cis13/trans11} (2.4%), as well as dimethyl acetals (DMA)/aldehydes (ALDE) probably derived from plasmalogens, C_{14:0} DMA (5.0%), C_{16:0} ALDE (3.8%), C_{16:1cis-9} DMA (1.1%), C_{16:0} DMA (15.3%), C_{18:1cis11} DMA (0.8%) [35]. No data are available on polar lipid, quinone or other cell wall/envelope components that may be taxonomically significant

Taxonomic perspective

The data presented in Figure 1, based on an evaluation of the 16S rRNA gene sequence data provide an interesting insight into the nomenclature and classification of members of the genus *Spirochaeta*. In determining which species currently placed in this genus should remain members of this genus it is important to note that the primary criterion is which species group with the type strain of the type species of the genus *Spirochaeta*. It should be noted that the type species of this genus is *Spirochaeta plicatilis* and only a description serves as the type since no type strain appears to be available. This makes it difficult to determine which species represented by living type strains belong within the genus *Spirochaeta*. This is important because the monophyletic group delineated by the majority of the members of the genus *Spirochaeta* and members of the genus *Borrelia* does not split into two monophyletic groups corresponding with the members of the genus *Spirochaeta* and *Borrelia*, but causes the members of the genus *Spirochaeta* to appear to be paraphyletic. If one of the goals of modern taxonomy is to classify species in a single

genus only if the members of the genus constitute a monophyletic group, then there are three possible solutions. The first is that all members of the genus *Borrelia* should be transferred to the genus *Spirochaeta*, although this is also complicated by the fact that a type strain for the type species of the genus *Borrelia*, *Borrelia anserine* has never been designated. The second alternative would be to create a number of genera based on monophyletic groups to be found within the current analysis of members of the genus *Spirochaeta*. The third

alternative would be to accept the *status quo* whereby members of the genus *Spirochaeta* appear to constitute a paraphyletic group. However, a key factor in attempting to undertake such a reclassification would be the absence of type strains of the type species of the genera *Spirochaeta* and *Borrelia*. There are already indications that the evolutionary group constituting members of the genera *Spirochaeta* and *Borrelia* show an interesting degree of diversity at the level of morphology, physiology and the genome.

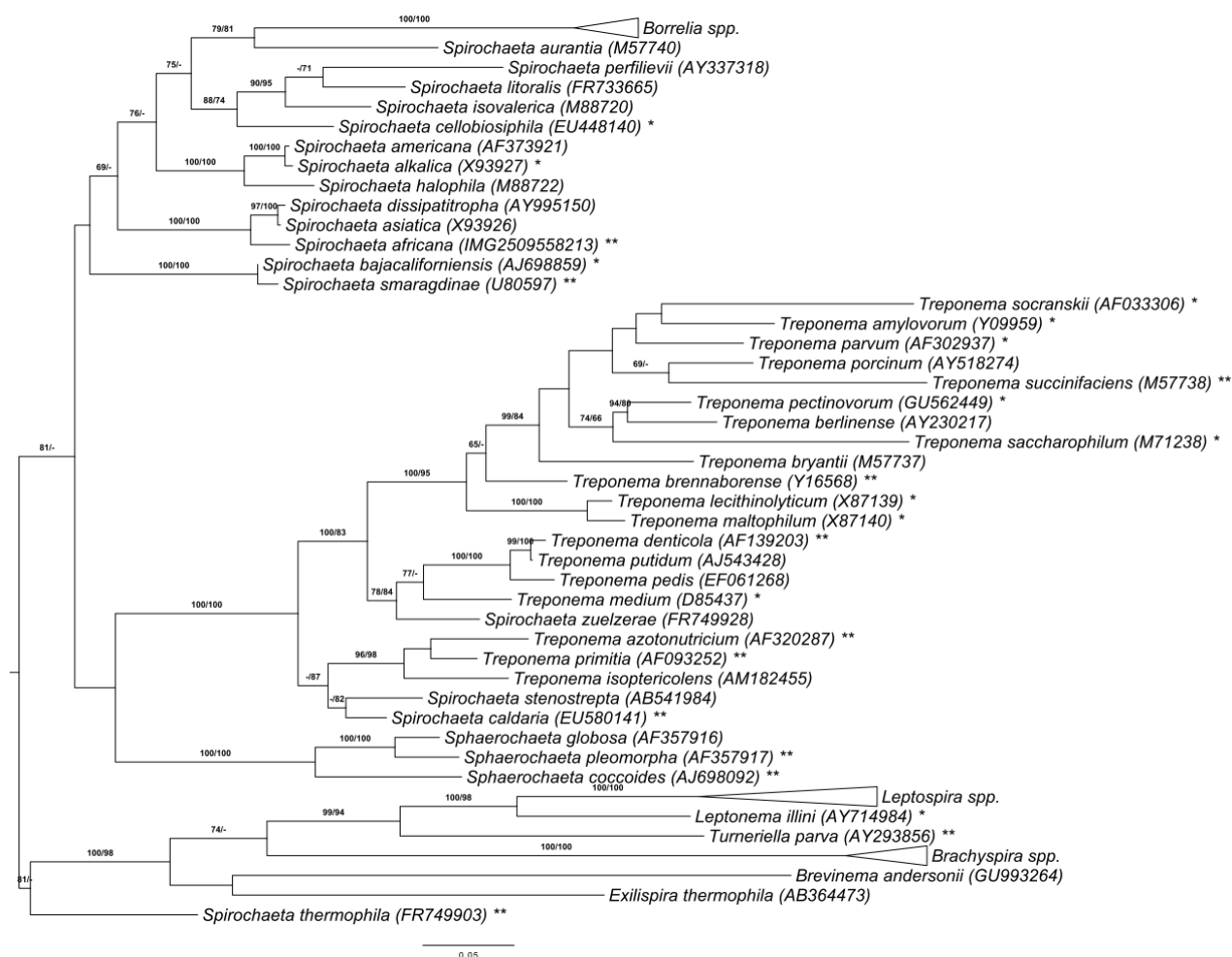


Figure 1. Phylogenetic tree highlighting the position of *S. africana* relative to the type strains of the other species within the phylum 'Spirochaetes'. The tree was inferred from 1,332 aligned characters [7,8] of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [9]. Rooting was done initially using the midpoint method [10] and then checked for its agreement with the current classification (Table 1). The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 350 ML bootstrap replicates [11] (left) and from 1,000 maximum-parsimony bootstrap replicates [12] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [13] are labeled with one asterisk, those also listed as 'Complete and Published' with two asterisks (see [14-20] and CP003155 for *Sphaerochaeta pleomorpha*, CP002903 for *Sphaerochaeta thermophila*, CP002696 for *Treponema brennaborens*, CP001841 for *T. azotonutricium* and CP001843 for *T. primitia*). Note: *Spirochaeta caldaria*, *S. stenostrepta* and *S. zuelzeri* were effectively renamed to *T. caldaria*, *T. stenostrepta* and *T. zuelzeri* in [15], however, the names have not yet been validly published.

Table 1. Classification and general features of *S. africana* Z-7692^T according to the MIGS recommendations [21] and the NamesforLife database [22].

MIGS ID	Property	Term	Evidence code
		Domain <i>Bacteria</i>	TAS [23]
		Phylum <i>Spirochaetae</i>	TAS [24,25]
		Class <i>Spirochaetes</i>	TAS [25,26]
	Current classification	Order <i>Spirochaetales</i>	TAS [27,28]
		Family <i>Spirochaetaceae</i>	TAS [27,29]
		Genus <i>Spirochaeta</i>	TAS [27,30-32]
		Species <i>Spirochaeta africana</i>	TAS [1]
		Type strain Z-7692	TAS [1]
	Gram stain	negative	TAS [1]
	Cell shape	spiral shaped	TAS [1]
	Motility	motile	TAS [1]
	Sporulation	none	TAS [1]
	Temperature range	mesophile	TAS [1]
	Optimum temperature	30 - 37°C	TAS [1]
	Salinity	halophile	TAS [1]
MIGS-22	Oxygen requirement	anaerobic, aerotolerant	TAS [1]
	Carbon source	saccharolytic, utilize carbohydrates	TAS [1]
	Energy metabolism	chemoorganotroph	TAS [1]
MIGS-6	Habitat	alkaline salt lakes, fresh water	TAS [1]
MIGS-15	Biotic relationship	free living	TAS [1]
MIGS-14	Pathogenicity	none	TAS [1]
	Biosafety level	1	TAS [33]
	Isolation	bacterial bloom in the brine under trona from alkaline lake	TAS [1]
MIGS-4	Geographic location	Lake Magadi (Kenya)	TAS [1]
MIGS-5	Sample collection time	1993 or before	NAS
MIGS-4.1	Latitude	-1.945	NAS
MIGS-4.2	Longitude	36.253	NAS
MIGS-4.3	Depth	not reported	
MIGS-4.4	Altitude	not reported	

Evidence codes - TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). Evidence codes are from the Gene Ontology project [34].



Figure 2. Scanning electron micrograph of *S. africana* strain Z-7692^T

Genome sequencing and annotation

Genome project history

This organism was selected for sequencing on the basis of its phylogenetic position [36,37], and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project [38]. The genome project is deposited in the Genomes On Line Database [13] and the complete genome sequence is deposited in GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI) using state of the art sequencing technology [39]. A summary of the project information is shown in Table 2.

Growth conditions and DNA isolation

S. africana strain Z-7692^T, DSM 8902, was grown anaerobically in DSMZ medium 700 (Alkaliphilic *Spirochaeta* medium) [40] at 37°C. DNA was isolated from 0.5-1 g of cell paste using MasterPure Gram-positive DNA purification kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer with modification st/LALM for cell lysis as described in Wu *et al.* 2009 [41]. DNA is available through the DNA Bank Network [42].

Table 2. Genome sequencing project information

MIGS ID	Property	Term
MIGS-31	Finishing quality	Finished
MIGS-28	Libraries used	Four genomic libraries: one 454 pyrosequence standard library, two 454 PE libraries (4 kb and 6 kb insert size), one Illumina library
MIGS-29	Sequencing platforms	Illumina GAii, 454 GS FLX Titanium
MIGS-31.2	Sequencing coverage	123.6 × Illumina; 23.4 × pyrosequence
MIGS-30	Assemblers	Newbler version 2.3-PreRelease-6/30/2009, Velvet 1.0.13, phrap version SPS - 4.244
MIGS-32	Gene calling method	Prodigal 1.4, GenePRIMP
	INSDC ID	CP003282
	GenBank Date of Release	April 2, 2012
	GOLD ID	Gc02193
	NCBI project ID	52939
	Database: IMG	2509276057
MIGS-13	Source material identifier	DSM 8902
	Project relevance	Tree of Life, GEBA

Genome sequencing and assembly

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [43]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly consisting of 511 contigs in one scaffold was converted into a phrap [44] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (459.3 Mb) was assembled with Velvet [45] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 234.5 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 21. The Phred/Phrap/Consed software package [44] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [43], Dupfinisher [46], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.-F. Chang, unpublished). A total of 132 additional reactions were necessary to close some gaps and to raise the quality of the final contigs. Illumina reads were also used to correct potential base errors and increase con-

sensus quality using a software Polisher developed at JGI [47]. The error rate of the final genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 480.9 x coverage of the genome. The final assembly contained 509,107 pyrosequence and 12,708,968 Illumina reads.

Genome annotation

Genes were identified using Prodigal [48] as part of the DOE-JGI genome annotation pipeline [20], followed by a round of manual curation using the JGI GenePRIMP pipeline [49]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [50].

Genome properties

The genome consists of a 3,285,855 bp long chromosome with a G+C content of 57.8% (Table 3 and Figure 3). Of the 2,874 genes predicted, 2,817 were protein-coding genes, and 57 RNAs; 35 pseudogenes were also identified. The majority of the protein-coding genes (74.2%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

Table 3. Genome Statistics

Attribute	Value	% of Total
Genome size (bp)	3,285,855	100.00%
DNA coding region (bp)	3,080,373	93.75%
DNA G+C content (bp)	1,898,112	57.77%
Number of replicons	1	
Extrachromosomal elements	0	
Total genes	2,874	100.00%
RNA genes	57	1.98%
rRNA operons	3	
Protein-coding genes	2,817	98.02%
Pseudo genes	35	1.22%
Genes with function prediction	2,133	74.22%
Genes in paralog clusters	1,205	41.93%
Genes assigned to COGs	2,153	74.91%
Genes assigned Pfam domains	2,235	77.77%
Genes with signal peptides	247	8.59%
Genes with transmembrane helices	847	29.47%
CRISPR repeats	1	

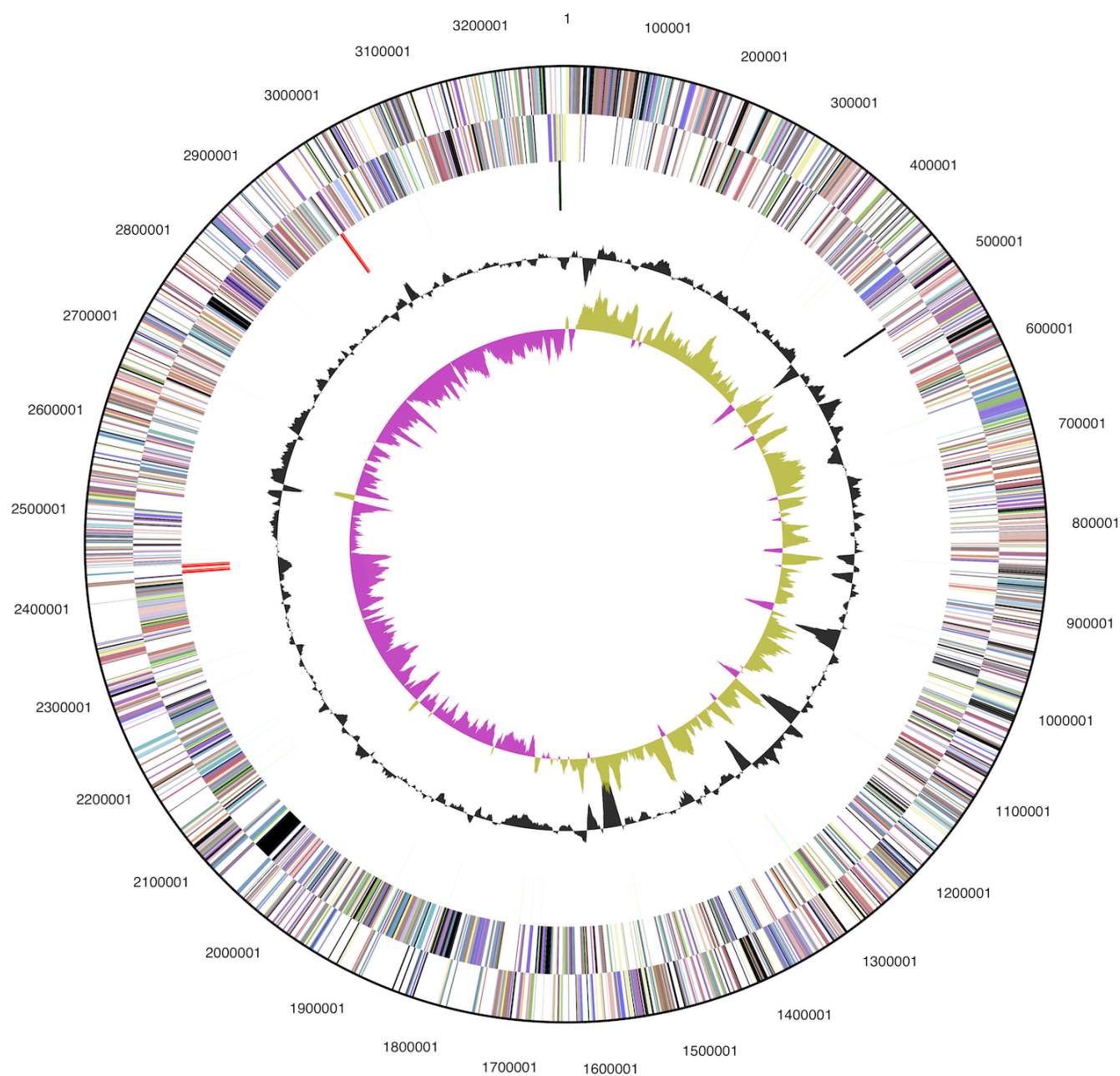


Figure 3. Graphical map of the chromosome. From outside to the center: Genes on forward strand (color by COG categories), genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content (black), GC skew (purple/olive).

Table 4. Number of genes associated with the general COG functional categories

Code	Value	%age	Description
J	151	6.4	Translation, ribosomal structure and biogenesis
A	0	0.0	RNA processing and modification
K	137	5.8	Transcription
L	138	5.8	Replication, recombination and repair
B	0	0.0	Chromatin structure and dynamics
D	24	1.0	Cell cycle control, cell division, chromosome partitioning
Y	0	0.0	Nuclear structure
V	49	2.1	Defense mechanisms
T	239	10.1	Signal transduction mechanisms
M	149	6.3	Cell wall/membrane/envelope biogenesis
N	93	3.9	Cell motility
Z	0	0.0	Cytoskeleton
W	1	0.0	Extracellular structures
U	60	2.5	Intracellular trafficking, secretion, and vesicular transport
O	100	4.2	Posttranslational modification, protein turnover, chaperones
C	109	4.6	Energy production and conversion
G	185	7.8	Carbohydrate transport and metabolism
E	179	7.5	Amino acid transport and metabolism
F	62	2.6	Nucleotide transport and metabolism
H	68	2.9	Coenzyme transport and metabolism
I	57	2.4	Lipid transport and metabolism
P	103	4.3	Inorganic ion transport and metabolism
Q	23	1.0	Secondary metabolites biosynthesis, transport and catabolism
R	267	11.3	General function prediction only
S	181	7.6	Function unknown
-	721	25.1	Not in COGs

Insights from the genome sequence

Phylogenomic analyses

According to the results from 16S rRNA gene analysis (Figure 1), for a comparative analysis the genome sequences of *S. africana* (GenBank ID CP003282), *S. alkalica* (GenBank ID PRJNA169743), *S. caldaria* (CP002868) and *S. smaragdinae* (CP002116) were used. The genomes of *S. caldaria* (3.2 Mb, 2,928 protein-coding genes), *S. africana* (3.3 Mb, 2,874 protein-coding genes) and *S. alkalica* (3.4 Mb, 2,938 protein-coding genes) have a similar size, whereas the genome of *S. smaragdinae* (4.7 Mb, 4,363 protein-coding gene) is significantly larger in size. *S. caldaria* and *S. smaragdinae* have similar G+C contents, 46% and 49%, respectively. The G+C con-

tents of *S. alkalica* and *S. africana* are significantly higher, 61% and 58%, respectively.

An estimate of the overall similarity between the genomes of *S. africana*, and those of the other *Spirochaeta* species was generated with the GGDC-Genome-to-Genome Distance Calculator [51,52]. This system calculates the distances by comparing the genomes to obtain HSPs (high-scoring segment pairs) and interfering distances from the set of formulas (1, HSP length / total length; 2, identities / HSP length; 3, identities / total length). Table 5 shows the results of the pairwise comparison.

The comparison of *S. africana* with *S. alkalica* reached the highest scores using the GGDC, 5.2% of the average of genome length are covered with HSPs. The identity within the HSPs was 86.4%, whereas the identity over the whole genome was 4.5%. Lower similarity scores were observed in the comparison of *S. africana* with *S. caldaria* and with *S. smaragdinae* only 1.62% and 1.64%, respectively, of the average of both genome lengths

are covered with HSPs. The identity within these HSPs was 84.5% and 83.5%, respectively, whereas the identity over the whole genome was only 1.4% in both comparisons. *S. alkalica* shows the highest GGDC scores with *S. smaragdinae*: 2.5% of the average of genome length are covered with HSPs and the identity within the HSPs was 87.7%, whereas the identity over the whole genome was 2.2% [51].

Table 5. Pairwise comparison of *S. africana* with *S. alkalica*, *S. caldaria*, and *S. smaragdinae*, using the GGDC-Genome-to-Genome Distance Calculator.

		HSP length / total length [%]	identities / HSP length [%]	identities / total length [%]
<i>S. africana</i>	<i>S. alkalica</i>	5.21	86.44	4.51
<i>S. africana</i>	<i>S. caldaria</i>	1.62	84.50	1.37
<i>S. africana</i>	<i>S. smaragdinae</i>	1.64	83.52	1.37
<i>S. smaragdinae</i>	<i>S. alkalica</i>	2.51	87.71	2.20
<i>S. smaragdinae</i>	<i>S. caldaria</i>	1.52	83.91	1.28
<i>S. caldaria</i>	<i>S. alkalica</i>	2.08	88.57	1.85

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Note

IJSEM will validate the names *T. caldarium*, *T. stenostreptum* and *T. zuelzeriae* in Validation List 153 (September 2013)

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